Antipsychotic agents differ in how fast they come off the dopamine D_2 receptors. Implications for atypical antipsychotic action

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Rationale and objective: While the blockade of dopamine D_2 receptors are necessary for antipsychotic action, antipsychotic agents differ nearly a thousand-fold in their affinity for the D_2 receptor. This affinity is determined by the rate at which the antipsychotic agent binds to (k_{on}) and the rate at which it dissociates from (k_{off}) the D_2 receptors. The objective of this study was to determine the relationship between k_{on}, k_{off} and the affinity (K_i) of antipsychotic agents for the D_2 receptors, with particular reference to typical and atypical antipsychotic agents. **Design:** The k_{off} of several typical as well as atypical antipsychotic agents (nemonapride, spiperone, haloperidol, chlorpromazine, raclopride, olanzapine, sertindole, clozapine and quetiapine) was measured in vitro using the ^{3}H-radiolabelled analogues of these drugs. The affinity of these drugs for the D_2 receptor was determined by competition with ^{3}H-raclopride in vitro. The k_{on} was derived from values of affinity and k_{off}. **Main outcome measures:** k_{on}, k_{off}, and the K_i of antipsychotic drugs. **Results:** The range of affinity values was similar to that conventionally accepted (0.025–155 nmol/L). The k_{off} values varied a thousand-fold from 0.002 to 3.013 min^{-1}, with relatively little variation in k_{on}. The rate at which antipsychotic agents come off the receptor (k_{off}) accounted for 99% of the variation in their affinity for the D_2 receptor; differences in k_{on} did not account for differences in affinity. **Conclusions:** The differences in the affinity of antipsychotic agents are entirely determined by how fast they come off the D_2 receptor. These differences in k_{off} may lead to functionally different kinds of dopamine blockade. Drugs with a higher k_{off} will be faster in blocking receptors, and once blocked, will provide more access to surges in dopamine transmission. Since atypical drugs show a lower affinity and a faster dissociation, a higher k_{off} for the D_2 receptor is proposed as a mechanism for "atypical" antipsychotic effect.

Justification et objectif : Même si le blocage des récepteurs dopaminergiques D_2 est nécessaire pour que les neuroleptiques agissent, l'affinité de ceux-ci pour le récepteur D_2 diffère d'un ordre de grandeur

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qui atteint presque le millier. Cette affinité est déterminée par la vitesse à laquelle le neuroleptique se fixe (k_{on}) aux récepteurs D_2 et à la vitesse à laquelle il s'en dissocie (k_{off}). L'étude visait à déterminer le lien entre les facteurs k_{on}, k_{off} et l'affinité (K_i) des neuroleptiques pour les récepteurs D_2 et plus particulièrement les neuroleptiques typiques et atypiques. **Conception :** On a mesuré le facteur k_{off} de plusieurs neuroleptiques typiques et atypiques (némonapride, spipérone, halopéridol, chlorpromazine, raclopride, olanzapine, sertindole, clozapine et quétiapine) in vitro en utilisant les analogues radiomarqués ^3H de ces médicaments. L'affinité de ces médicaments pour le récepteur D_2 a été dérivée par concurrence avec le ^3H-raclopride in vitro. On a dérivé la valeur k_{on} des valeurs de l'affinité et du facteur k_{off}. **Principales mesures de résultats :** Facteurs k_{on}, k_{off} et K_i des neuroleptiques. **Résultats :** La plage des valeurs d'affinité ressemblait à celle qui est acceptée habituellement (0,025–155 nmol/L). Les valeurs k_{off} ont varié d'un facteur de l'ordre de 1000, soit de 0,002 à 3,013 min^{-1}, et la valeur k_{on} a varié très peu. La vitesse à laquelle les neuroleptiques se dissocient du récepteur (k_{off}) explique 99 % de la variation de leur affinité pour le récepteur D_2. Les différences des valeurs k_{on} n'expliquaient pas les différences d'affinité. **Conclusions :** Les différences d'affinité des neuroleptiques sont entièrement fonction de la vitesse à laquelle ils se dissocient du récepteur D_2. Ces différences des valeurs k_{off} peuvent entraîner des types différents, sur le plan fonctionnel, de blocage de la dopamine. Les médicaments qui ont une valeur k_{off} plus élevée bloqueront plus rapidement les récepteurs et, après le blocage, assureront un accès plus important aux pics de transmission de la dopamine. Comme les médicaments atypiques ont une affinité moindre et se dissocient plus rapidement, on propose une valeur k_{off} plus élevée pour le récepteur D_2 comme mécanisme d'effet neuroleptique «atypique».

Introduction

All currently used antipsychotic agents bind to dopamine D_2 receptors, as assessed by their "affinity" or "potency" for the D_2 receptor in vitro.[1,2] Discussions of the D_2 effects of antipsychotic agents often use the term "affinity" in a pharmacologic context and "potency" in a clinical context. Both of these terms usually refer to the equilibrium dissociation constant, K_d, or to the related term K_i (which represents the equilibrium dissociation constant measured by competitive inhibition). However, K_d or K_i are hybrid parameters, reflecting the situation at equilibrium. These parameters are derived, as shown in the equation below, from 2 more elemental parameters that characterize the dynamic essence of drug-receptor interaction. The binding of a drug to a single receptor is said to obey the simple mass action law and can be represented as below:[3,4]

$$D + R \underset{koff}{\overset{kon}{\rightleftharpoons}} DR; \text{ while } Kd = \frac{koff}{kon}$$

In this formulation the rate at which a drug (D) binds to a receptor (R) is determined by the concentration of the drug, the receptor and the association rate constant, k_{on} (unit concentration^{-1} time^{-1}, also called on-rate constant). The rate at which the drug-receptor complex DR dissociates is determined by the concentration of the complex and the dissociation rate constant, k_{off} (unit time^{-1}, also called off-rate constant). K_d, the equilibrium constant, which equals k_{off}/k_{on}, allows one to predict only the equilibrium state of the reaction. On the other hand, rate constants k_{on} and k_{off} allow one to predict not only the equilibrium, but also how fast the drug-receptor system responds to perturbations in the concentration of the drug or another competitor. Since the endogenous dopamine levels are not static and are known to show transient 10-fold increases,[5] k_{on} and k_{off} are more relevant parameters for understanding dynamic drug action.

We were interested in this issue because of the recent findings that atypical antipsychotics are particularly responsive to sudden increases in endogenous dopamine and this may confer on them unique clinical properties.[6,7] Since it is k_{on} and k_{off} that determine how a drug responds to sudden changes in concentration and competition, we were interested in determining the k_{on} and k_{off} of antipsychotic agents. In theory, a difference in either k_{on} or k_{off}, or both, can be responsible for changes in affinity, and in practice that seems to be the case. For example, atropine has an affinity twice that of methylatropinium for the cholinergic receptors; this difference in K_d is driven mainly by differences in their k_{on} with very similar k_{off} values.[4] On the other hand, the 100-fold differences in affinity among β-blockers are largely owing to differences in k_{off} in the face of relatively similar k_{on} rates.[8] Thus, it remains to be established whether k_{on} or k_{off}, or both, contribute to the differential affinity of antipsychotics for the D_2 receptors. To our knowledge this issue has never been systematically addressed.

Methods

The aim of this experiment was to determine the k_{on} and k_{off} of a series of antipsychotics and relate them to their more commonly measured parameter, K_i, the inhibition constant. As shown in equation 1, since the 3 parameters are related, determination of any 2 permits the delineation of the third. Of these, the k_{off} and the K_i can be determined with greatest accuracy; therefore we chose to measure these 2 and obtain k_{on} as a result.[9]

Tissue

Rat brains were obtained from Pel-Freez (Rogers, Ark.). The striata were dissected in the frozen state and homogenized in buffer (50 mmol/L TRIS-HCl, 1 mmol/L EDTA, 5 mmol/L KCl, 1.5 mmol/L $CaCl_2$, 4 mmol/L $MgCl_2$, 120 mmol/L NaCl; pH 7.4) using a Brinkmann Polytron homogenizer PT-10 (Brinkmann Scientific, Westbury, NY) (5 seconds at setting 5). Pooled tissue from several rats was used for the determination of k_{off}.

Measurement of k_{off}

Two methods are widely used to determine k_{off}.[3,4] Both methods rely on measuring the rate of dissociation of the radiolabelled ligand over time. In 1 method, dissociation is initiated by instantaneous dilution, which obviates any reassociation (the "dilution" method).[3,4] In the other method, dissociation is measured by the addition of an excess of another antagonist, which competes overwhelmingly for the same receptor and thereby obviates any reassociation ("excess raclopride" method).[9] If the receptor–ligand interaction is simply a first-order reaction, as characterized by equation 1, then the 2 methods should give identical results. On the other hand, if rebinding or cooperativity are prominent, the interaction would result in a deviation from first-order kinetics, and under these conditions the 2 methods may give deviant results.[3,4]

Dilution method

At room temperature for 60 minutes, 1 mL of [^{3}H]antipsychotic drug and 1 mL of rat striatal tissue (final = 2 mg tissue/mL) were incubated to achieve the final antipsychotic concentrations listed below. After 1 hour, 16 mL of buffer was added, and the resulting mixture was stirred to provide instantaneous dilution. Eight aliquots of 2 mL of the resulting suspension were removed and rapidly filtered at various times at room temperature. The aliquots were filtered under vacuum through pre-soaked glass fibre filters (Whatman GF/B; Brandel, Gaithersburg, Md.) using a Millipore (Millipore, Bedford, Mass.) filter manifold. After washing the filters rapidly with 5 mL of buffer, they were placed in scintillation minivials (Packard, Chicago) and were monitored for tritium 6 hours later in a Packard 4660 scintillation spectrometer at 55% efficiency. In a parallel set of tubes, nonspecific binding of the [^{3}H]antipsychotic drug was determined in the presence of 10 µmol/L S-sulpiride. Each antipsychotic was tested on 2 or 3 separate occasions.

Excess raclopride method

In the presence of the [^{3}H]antipsychotic drug, 18 mL of buffer was prepared containing a total of 2 mg of rat striatal tissue to obtain the final concentration listed below. After 1 hour of incubation at room temperature, 0.5 mL of raclopride was added to give a final concentration of 10 µmol/L raclopride. Aliquots of 2 mL of the suspension were filtered and counted as in the dilution method. In a parallel set of tubes, nonspecific binding of the [^{3}H]antipsychotic drug was done in the presence of 10 µmol/L S-sulpiride. Each antipsychotic was tested on 2 separate occasions, the results were reliable, and the averaged data are presented.

The final concentration of each [^{3}H]antipsychotic drug in the 2-mL pre-incubate (dilution method) and in the 18-mL pre-incubate (excess raclopride method) were identical. These concentrations, chosen to approximate the free molarities in the patients' spinal fluid or plasma water phase, were: [^{3}H]nemonapride (100 Ci/mmol; New England Nuclear, Boston), 0.2 nmol/L; [^{3}H]spiperone (Amersham), 0.25 nmol/L; [^{3}H]haloperidol (12 Ci/mmol; New England Nuclear), 4 nmol/L; [^{3}H]raclopride (79 Ci/mmol; New England Nuclear), 2 nmol/L; [^{3}H]sertindole (47 Ci/mmol; H. Lundbeck A/S, Copenhagen-Valby, Denmark), 5 nmol/L; [^{3}H]chlorpromazine (25 Ci/mmol; New England Nuclear), 3 nmol/L; [^{3}H]olanzapine (81 Ci/mmol; Lilly Research Laboratories, Indianapolis), 5 nmol/L; [^{3}H]clozapine (84 Ci/mmol; New England Nuclear), 10 nmol/L in the presence of 300 nmol/L clozapine; [^{3}H]quetiapine (14 Ci/mmol, custom-prepared by New England Nuclear), 10 nmol/L in the presence of 200 nmol/L quetiapine. The time intervals over which the 8 measurements were distributed were different for dif-

ferent antipsychotics and were decided on the basis of preliminary experiments to provide an optimal estimate of rate of dissociation (e.g., samples every 10 seconds for [^{3}H]clozapine to every 30 minutes for [^{3}H]nemonapride). In each case the specific binding at time zero was taken as 100%, and the effect of dilution over time was plotted to obtain the time for 50% decline in binding ($t_{1/2}$). Since $t_{1/2} = 0.693/k_{off}$, k_{off} was determined from the measured $t_{1/2}$ as $0.693/t_{1/2}$.

Measurement of K_i

The details of the method for the determination of K_i have been presented in detail previously.[2] Briefly, the long form of the D_2 receptor was stable expressed in GH4Cl cells.[10] Cells were collected and suspended without washing or centrifugation and were homogenized (5 seconds at setting 5 in a Brinkmann Polytron homogenizer PT-10) to yield approximately 200 ug/mL protein. [^{3}H]raclopride, the membrane suspension and unlabelled antipsychotic of interest were coincubated in 1.5 mL buffer with the final concentration of raclopride fixed at 2 nmol/L, the receptor concentration at 10 pmol/L and with varying concentrations of the antipsychotic for which the K_i was to be determined. The K_i was calculated from the IC_{50} concentrations using the Cheng–Prusoff formula[11] and a value of raclopride K_d of 1.6 nmol/L, obtained as described previously.[2]

Results

The detailed results are presented in Table 1 and Fig. 1. We did not find any significant differences (paired *t*-test, $t_{df\,7} = 0.925$, $p = 0.382$) between the $t_{1/2}$ determined using the dilution method or using the excess raclopride method. The results were highly correlated (Pearson's correlation coefficient 0.98, $p < 0.0001$); therefore for further calculations we pooled the data from these 2 methods.

The k_{off} values of the available antipsychotics varied almost 3 orders of magnitude, from a very slow dissociation constant of 0.0024 min^{-1} for nemonapride to 3.1 min^{-1} for quetiapine. The k_{on} values showed relatively less variation, from 10.6 nmol/L^{-1} min^{-1} for nemonapride to 166 nmol/L^{-1}min^{-1} for olanzapine. The K_i values were consistent with those reported previously.

Most importantly, the K_i values were very highly predicted by k_{off} ($F_{1,7} = 1656$, $p < 0.0001$) (Fig. 1), and showed no significant relationship with k_{on} values ($F_{1,7} = 0.05$, $p = 0.829$). The differences in the k_{off} of the antipsychotics explained 99% of the variance in their affinity for the D_2 receptors, whereas differences in k_{on} do not meaningfully relate to differences in affinity.

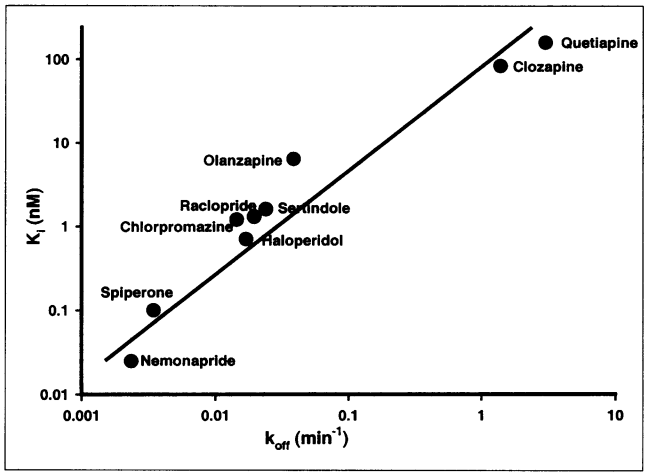

Fig. 1: The relationship between the equilibrium constant K_i (units of concentration) and the dissociation rate constant k_{off} (units of time) for a series of antipsychotics.

Table 1: Results of the determination of the affinity of antipsychotic agents for dopamine D_2 receptors

Antipsychotic agent	K_i, nmol/L	$t_{1/2}$ by the dilution method, min	$t_{1/2}$ by the excess raclopride method, min	k_{off}, min^{-1}	k_{on}, nmol/L^{-1} min^{-1}
Nemonapride	0.025	355	250	0.002	10.6
Spiperone	0.1	200	200	0.003	28.9
Haloperidol	0.7	40	42	0.017	41.4
Sertindole	1.2	47	49	0.014	83.1
Chlorpromazine	1.3	35	36	0.020	66.6
Raclopride	1.6	28	30	0.024	66.9
Olanzapine	6.4	18	18	0.039	166.2
Clozapine	82	0.5	0.5	1.386	59.2
Quetiapine	155	0.23	0.23	3.013	51.4

Discussion

Antipsychotics vary 3 orders of magnitude in their affinity (K_d or K_i) for the D_2 receptor. The data presented here demonstrate that the differences among antipsychotics are mainly owing to the rate at which they come off the receptor.

Our study is limited to the 9 antipsychotics tested. This limitation was imposed by the number of radiolabelled antipsychotics available to us. Fortunately, we were able to access most of the labelled antipsychotics, and they spanned a range of affinity from 0.025 nmol/L to 155 nmol/L and belonged to a range of different chemical classes (phenothiazines, butyrophenones, substituted benzamides, dibenzazepines, dibenzoxazepines), thus providing generalizability for these results.

A second limitation pertains to the extrapolation of kinetic results obtained in vitro to the in vivo situation. Our finding that the dilution estimates were no different from the excess-raclopride estimates reinforces the fact that the receptor homogenates and the drug behave under simple bimolecular assumptions in vitro.[4] However, in vivo the on-rate and the off-rate may be influenced by a number of conditions. The access of the drug to the receptor via blood flow or the passive-diffusion limitations may exert additional constraints on the rate of association beyond that of the parameter k_{on}. Similarly, local conditions such as endogenous dopamine competition, binding to spare receptors and rebinding after dissociation, as well as the modulating effect of other receptors could also alter the binding characteristics of drugs to the D_2 receptor.[12–14] Although it is likely that the precise value of these parameters will differ in vivo, the general principle identified herein should be applicable in vivo.[12]

These findings have interesting implications for understanding the differences between antipsychotic agents. One of the most comprehensive surveys of the receptor-binding properties of antipsychotics was by Meltzer et al,[15] who examined the binding of 37 atypical or presumed atypical antipsychotics on dopamine D_1 and D_2 as well as serotonin 5-HT_2 receptors. This paper is usually cited in support of the serotonin–dopamine hypothesis. But it is very important to note that Meltzer et al reported no differences in the serotonin affinities of typical versus atypical antipsychotics (pK_i values for 5-HT_2 affinity: 8.37 v. 8.36). They also found no difference in D_1 receptor binding either. The only major difference between typical and atypical antipsychotics was in their affinity for the D_2 receptor. The typical antipsychotic agents showed a much higher affinity for the D_2 receptor (pK_i 8.87 v. 7.01; $p < 0.001$) than atypical antipsychotic agents. The point here being that it is *not* the high 5-HT_2 affinity but the low D_2 affinity that makes an antipsychotic agent atypical.

This finding poses an interesting challenge. Antipsychotic agents are used clinically in doses that are inversely proportional to their affinity. This fact remains true even in the case of the newer atypical antipsychotic agents. For example, the relative in vitro affinities of haloperidol, risperidone, olanzapine and clozapine for the D_2 receptor are 1.5:3:17:150 nmol/L, with haloperidol being most potent and clozapine the least.[16] As predicted by these in vitro affinities, the clinical doses also share a similar relation — haloperidol 2 to 4 mg/d:risperidone 3 to 6 mg/d:olanzapine 10 to 20 mg/d:clozapine 250 to 450 mg/d. At first sight it may appear that giving 100 times more of a drug with a 100-times lower affinity should equate all things. Although giving a proportionally higher dose of a low-affinity antipsychotic agent may lead to equal occupancy at equilibrium (since equilibrium occupancy is based only on dose and affinity), the behaviour of these drugs under dynamic circumstances will still be very different. This is where the differences in k_{off} are crucial. We illustrate the importance of k_{off} by considering some dynamic circumstances.

When the concentration of a drug is increased it tends toward a higher occupancy. However, the rate at which the drugs move toward higher occupancy differs. The rate does not depend on affinity, but on the rate constants k_{on} and k_{off}. The time to reach this new equilibrium occupancy is *inversely* proportional to ($k_{on} \times$ concentration + k_{off})[3,4] in situations where there is no drop in the concentration of the drug due to the act of receptor-binding. By substituting values from Table 1, one finds that 310 nmol/L of clozapine will reach a higher occupancy equilibrium *100 times faster* than 4 nmol/L haloperidol. On the other hand, the rate at which a drug comes off the receptors, either when its concentration decreases or when there is competition from endogenous dopamine, is determined by k_{off} alone. From values in Table 1, one would expect 310 nmol/L of clozapine to come off the D_2 receptor nearly a *100 times faster* than 4 nmol/L haloperidol, a finding that we have empirically demonstrated in vitro before (unpublished data, 1999).

The higher dose of the agents with lower K_d, rather than equating the dynamic differences, actually accentuates them. Since the drugs with a higher k_{off} are given

in much higher doses, they speed up the rate at which the drugs increase their occupancy as already shown. Thus, one prediction of our finding would be that antipsychotic agents with a lower affinity, higher k_{off} and a faster half-time (toward the clozapine end of the spectrum) will be faster in occupying receptors *and* will be more responsive to endogenous changes in dopamine levels than antipsychotics having a lower k_{off} and slower half-time (toward the haloperidol end of the spectrum).

How these receptor kinetic differences translate into clinical differences is yet to be determined. But, it should be noted that low k_{off} antipsychotic agents (e.g., spiperone, nemonapride, haloperidol) have all been associated with extrapyramidal side effects (EPS) and prolactin elevation, whereas the high k_{off} antipsychotic agents (e.g., clozapine, quetiapine) are known to be free of EPS and prolactin elevation, essential features of an atypical antipsychotic ageny. This is observed even though the drugs are given in doses that (based on K_d) should have equivalent effects. We propose that it is the property of a high k_{off} at the D_2 receptor that makes antipsychotic agents more responsive to endogenous dopamine and hence less likely to give rise to side effects such as EPS and prolactin elevation, which are commonly associated with dopamine antagonism (unpublished data, 1999).[6] Thus, a high k_{off} at the D_2 receptor may be a mechanism for "atypical" antipsychotic effect.

We have shown that the variation in the affinity/potency or K_d/K_i of antipsychotic agents for D_2 receptors is almost entirely accounted for by their k_{off}. Antipsychotic agents differ almost a thousand-fold in the rate at which they come off the D_2 receptor. Since it is k_{off} that determines how quickly the antipsychotic drug will respond to the dynamic interaction between dopamine and D_2 receptors in the synapse, future research needs to explore the functional consequences of these differences between k_{off}.

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